silica); Maoz and Sagiv, *Langmuir*, **3**, 1034 (1987) (silanes on silica); Wasserman et al., *Langmuir*, **5**, 1074 (1989) (silanes on silica); Eltekova and Eltekov, *Langmuir*, **3**, 951 (1987) (aromatic carboxylic acids, aldehydes, alcohols and methoxy groups on titanium dioxide and silica); Lec et al., *J. Phys. Chem.*, **92**, 2597 (1988) (rigid phosphates on metals).

Each nanoparticle will have a plurality of oligonucleotides attached to it. As a result, each nanoparticle-oligonucleotide conjugate can bind to a plurality of oligonucleotides or nucleic acids having the complementary sequence.

Oligonucleotides of defined sequences are used for a variety of purposes in the practice of the invention. Methods of making oligonucleotides of a predetermined sequence are well-known. See, *e.g.*, Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual* (2nd ed. 1989) and F. Eckstein (ed.) *Oligonucleotides and Analogues*, 1st Ed. (Oxford University Press, New York, 1991). Solid-phase synthesis methods are preferred for both oligoribonucleotides and oligodeoxyribonucleotides (the well-known methods of synthesizing DNA are also useful for synthesizing RNA). Oligoribonucleotides and oligodeoxyribonucleotides can also be prepared enzymatically.

The invention provides methods of detecting nucleic acids. Any type of nucleic acid may be detected, and the methods may be used, *e.g.*, for the diagnosis of disease and in sequencing of nucleic acids. Examples of nucleic acids that can be detected by the methods of the invention include genes (*e.g.*, a gene associated with a particular disease), viral RNA and DNA, bacterial DNA, fungal DNA, cDNA, mRNA, RNA and DNA fragments, oligonucleotides, synthetic oligonucleotides, modified oligonucleotides, single-stranded and double-stranded nucleic acids, natural and synthetic nucleic acids, etc. Thus, examples of the uses of the methods of detecting nucleic acids include: the diagnosis and/or monitoring of viral diseases (*e.g.*, human immunodeficiency virus, hepatitis viruses, herpes viruses, cytomegalovirus, and Epstein-Barr virus), bacterial diseases (*e.g.*, tuberculosis, Lyme disease, *H. pylori*, *Escherichia coli* infections, *Legionella* infections, *Mycoplasma* infections, *Salmonella* infections), sexually transmitted diseases (*e.g.*, gonorrhea), inherited disorders (*e.g.*, cystic fibrosis, Duchene muscular dystrophy, phenylketonuria, sickle cell anemia), and

cancers (*e.g.*, genes associated with the development of cancer); in forensics; in DNA sequencing; for paternity testing; for cell line authentication; for monitoring gene therapy; and for many other purposes.

The methods of detecting nucleic acids based on observing a color change with the naked eye are cheap, fast, simple, robust (the reagents are stable), do not require specialized or expensive equipment, and little or no instrumentation is required. This makes them particularly suitable for use in, *e.g.*, research and analytical laboratories in DNA sequencing, in the field to detect the presence of specific pathogens, in the doctor's office for quick identification of an infection to assist in prescribing a drug for treatment, and in homes and health centers for inexpensive first-line screening.

The nucleic acid to be detected may be isolated by known methods, or may be detected directly in cells, tissue samples, biological fluids (*e.g.*, saliva, urine, blood, serum), solutions containing PCR components, solutions containing large excesses of oligonucleotides or high molecular weight DNA, and other samples, as also known in the art. See, *e.g.*, Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual* (2nd ed. 1989) and B.D. Hames and S.J. Higgins, Eds., *Gene Probes 1* (IRL Press, New York, 1995). Methods of preparing nucleic acids for detection with hybridizing probes are well known in the art. See, *e.g.*, Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual* (2nd ed. 1989) and B.D. Hames and S.J. Higgins, Eds., *Gene Probes 1* (IRL Press, New York, 1995).

If a nucleic acid is present in small amounts, it may be applied by methods known in the art. See, *e.g.*, Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual* (2nd ed. 1989) and B.D. Hames and S.J. Higgins, Eds., *Gene Probes 1* (IRL Press, New York, 1995). Preferred is polymerase chain reaction (PCR) amplification.

One method according to the invention for detecting nucleic acid comprises contacting a nucleic acid with one or more types of nanoparticles having oligonucleotides attached thereto. The nucleic acid to be detected has at least two portions. The lengths of these portions and the distance(s), if any, between them are chosen so that when the oligonucleotides on the nanoparticles hybridize to the nucleic acid, a detectable change

occurs. These lengths and distances can be determined empirically and will depend on the type of particle used and its size and the type of electrolyte which will be present in solutions used in the assay (as is known in the art, certain electrolytes affect the conformation of nucleic acids).

Also, when a nucleic acid is to be detected in the presence of other nucleic acids, the portions of the nucleic acid to which the oligonucleotides on the nanoparticles are to bind must be chosen so that they contain sufficient unique sequence so that detection of the nucleic acid will be specific. Guidelines for doing so are well known in the art.

Although nucleic acids may contain repeating sequences close enough to each other so that only one type of oligonucleotide-nanoparticle conjugate need be used, this will be a rare occurrence. In general, the chosen portions of the nucleic acid will have different sequences and will be contacted with nanoparticles carrying two or more different oligonucleotides, preferably attached to different nanoparticles. An example of a system for the detection of nucleic acid is illustrated in Figure 2. As can be seen, a first oligonucleotide attached to a first nanoparticle has a sequence complementary to a first portion of the target sequence in the single-stranded DNA. A second oligonucleotide attached to a second nanoparticle has a sequence complementary to a second portion of the target sequence in the DNA. Additional portions of the DNA could be targeted with corresponding nanoparticles. See Figure 17. Targeting several portions of a nucleic acid increases the magnitude of the detectable change.

The contacting of the nanoparticle-oligonucleotide conjugates with the nucleic acid takes place under conditions effective for hybridization of the oligonucleotides on the nanoparticles with the target sequence(s) of the nucleic acid. These hybridization conditions are well known in the art and can readily be optimized for the particular system employed. See, *e.g.*, Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual* (2nd ed. 1989). Preferably stringent hybridization conditions are employed.

Faster hybridization can be obtained by freezing and thawing a solution containing the nucleic acid to be detected and the nanoparticle-oligonucleotide conjugates. The solution